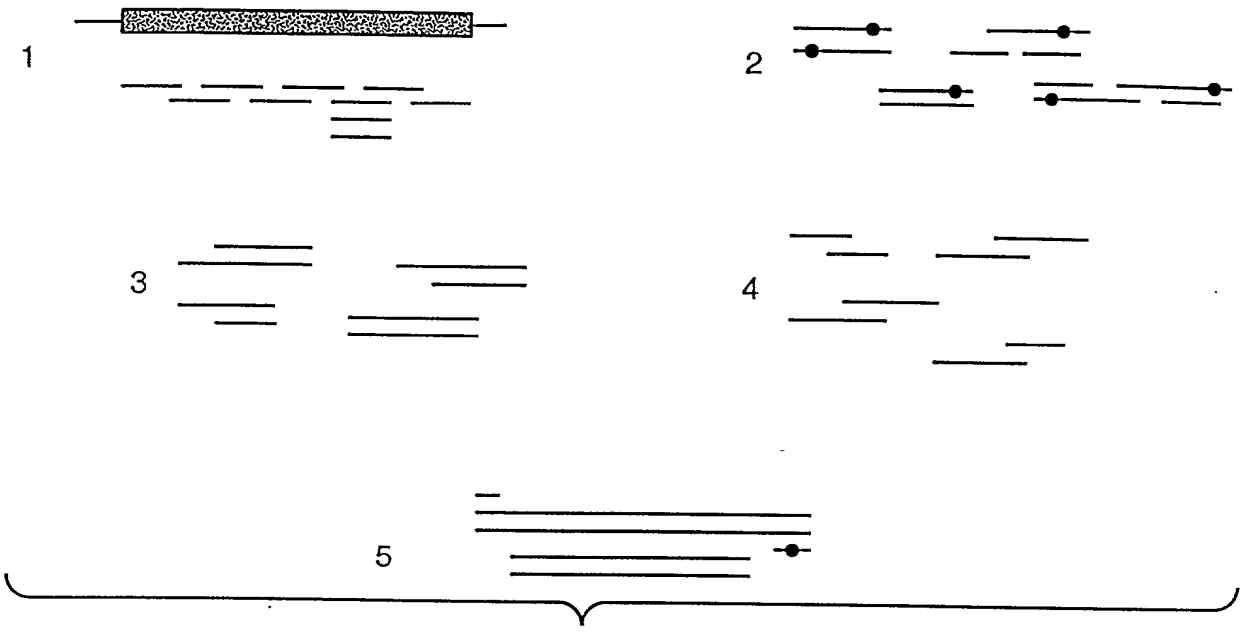


FIG._1

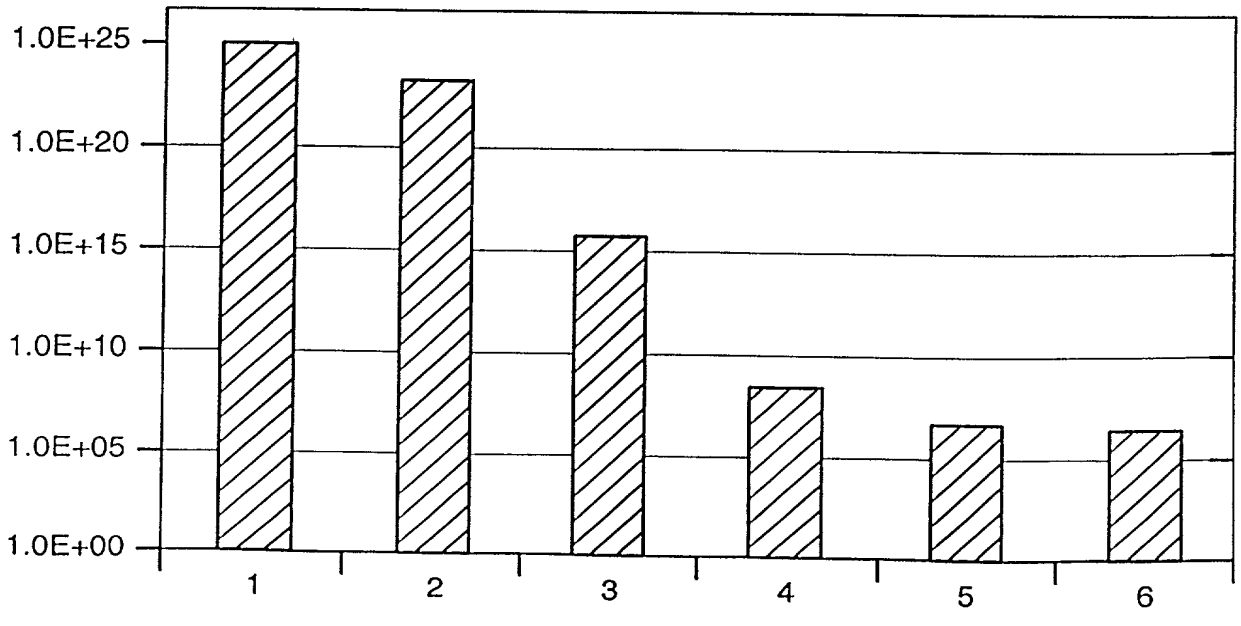


FIG._2

FIG._3

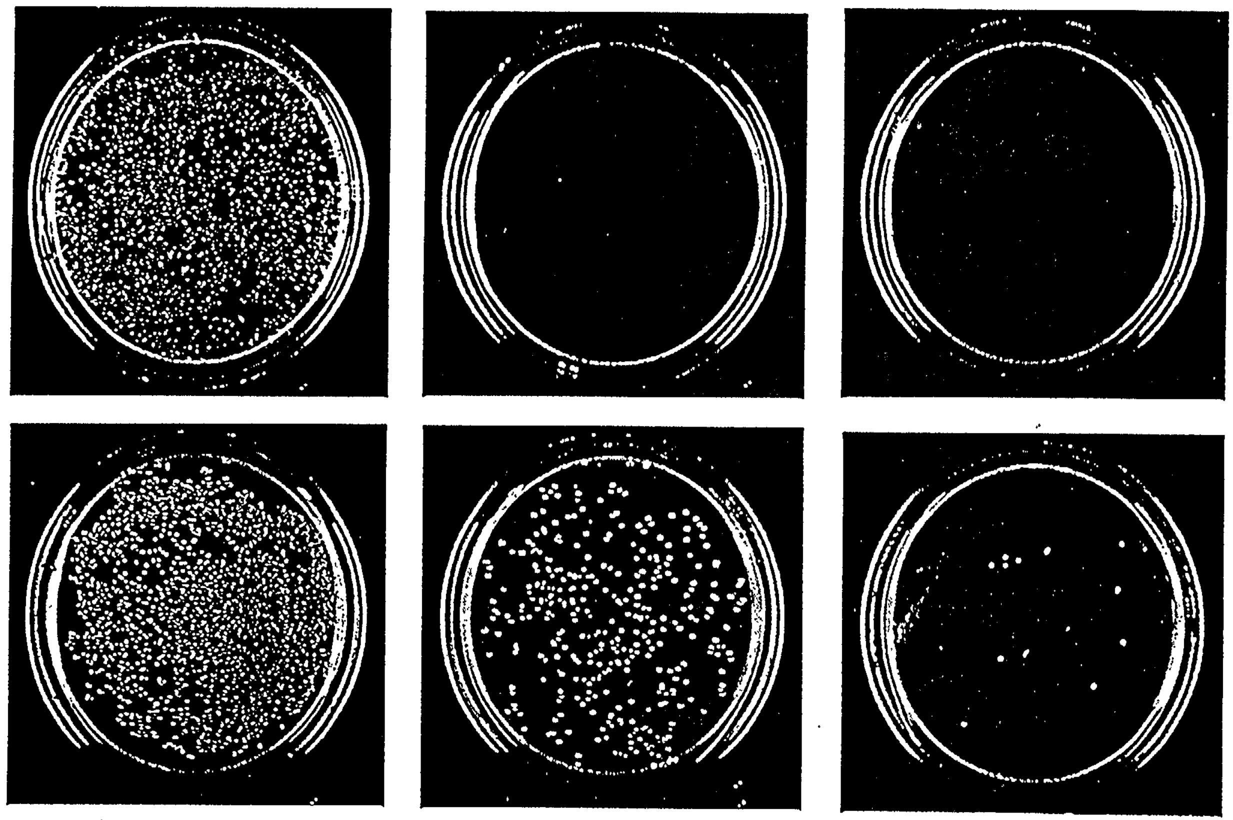
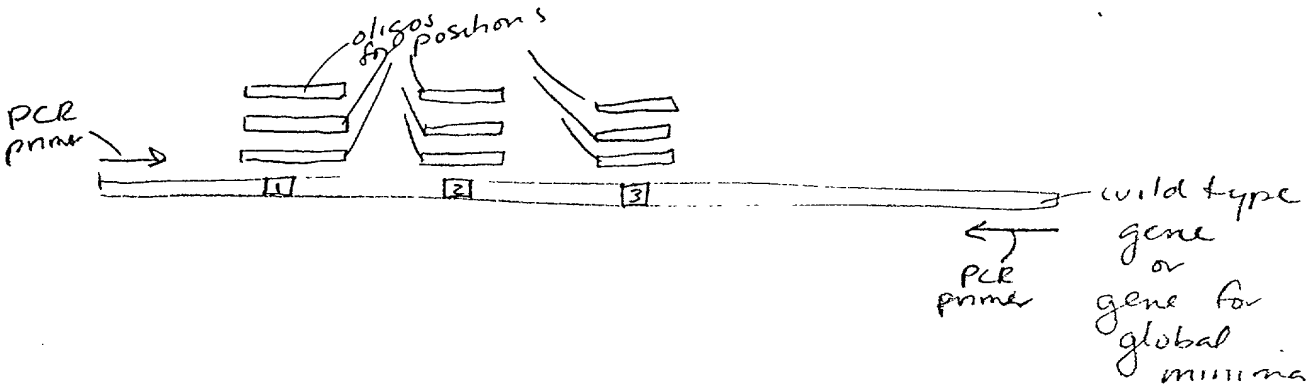
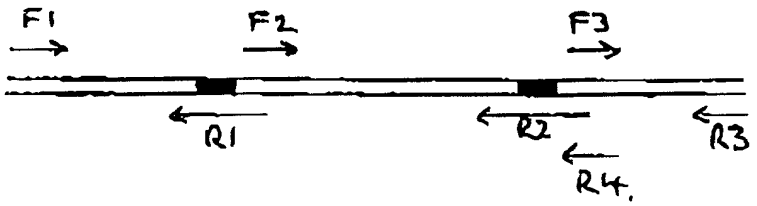


FIG._4

Figure 5



DIAGRAM



black box = region to be mutated.

Step1: Set up 3 PCR reactions.

Products:

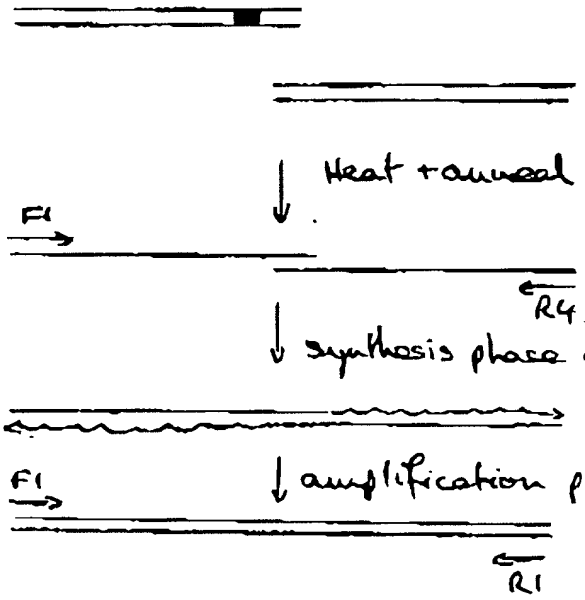
Fig 6A

Tube1:

Tube2:

Tub 3:

Step2. Set up PCR reaction with products of tube 1 + products tube 2 + F1 + R4

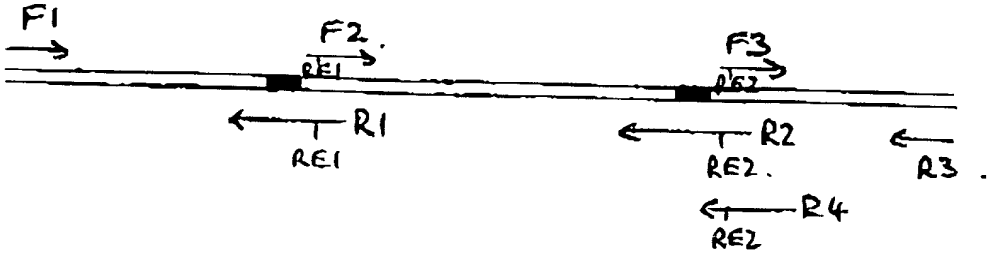


Step 3.

Repeat step 2 using product from step 2 + product from Step1, tube 3 + primers F1 or R3.

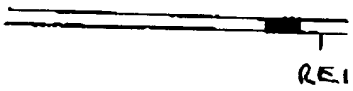
Fig 6B

DIAGRAM 2 Fig 7A

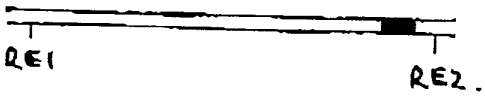


Step1 Set up 3 PCR reactions:

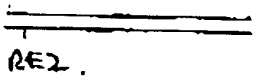
Tube1:



Tube 2:



Tube 3:



Step2: digest products from Step 1 with suitable restriction endonucleases

Step3: ligate digested product from Step2, tube2 with digested product from Step2, tube1.

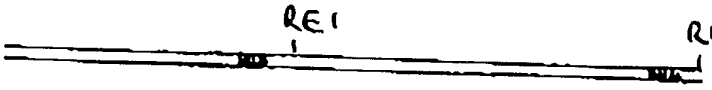
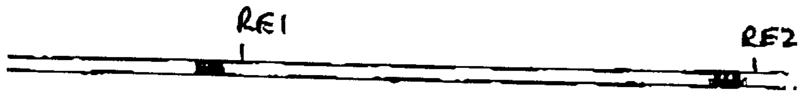


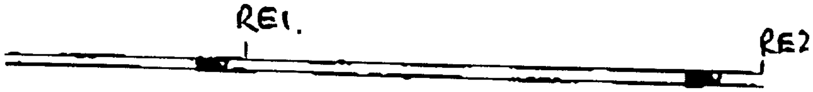
Fig 7B

Step 4

Amplify / via PCR ligated products of Step 3 with F1 + R4.

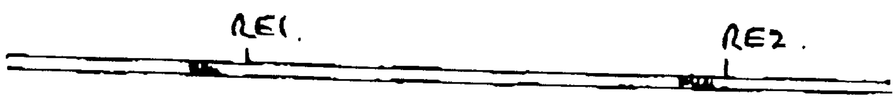


Step 5. Digest amplified product of step 4 with restriction endonuclease #2.



Step 6.

Ligate product from Step 5 with product from Step 2, tube 1.



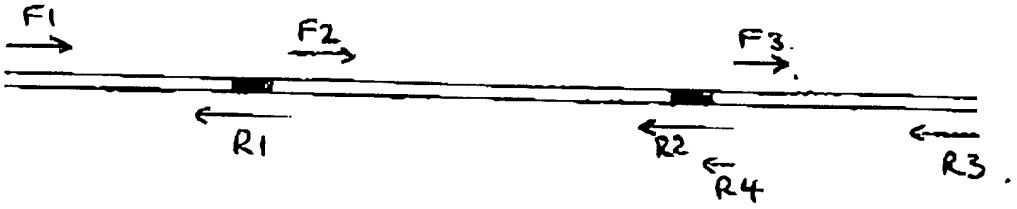
Step 7

Amplify product from step 6 with

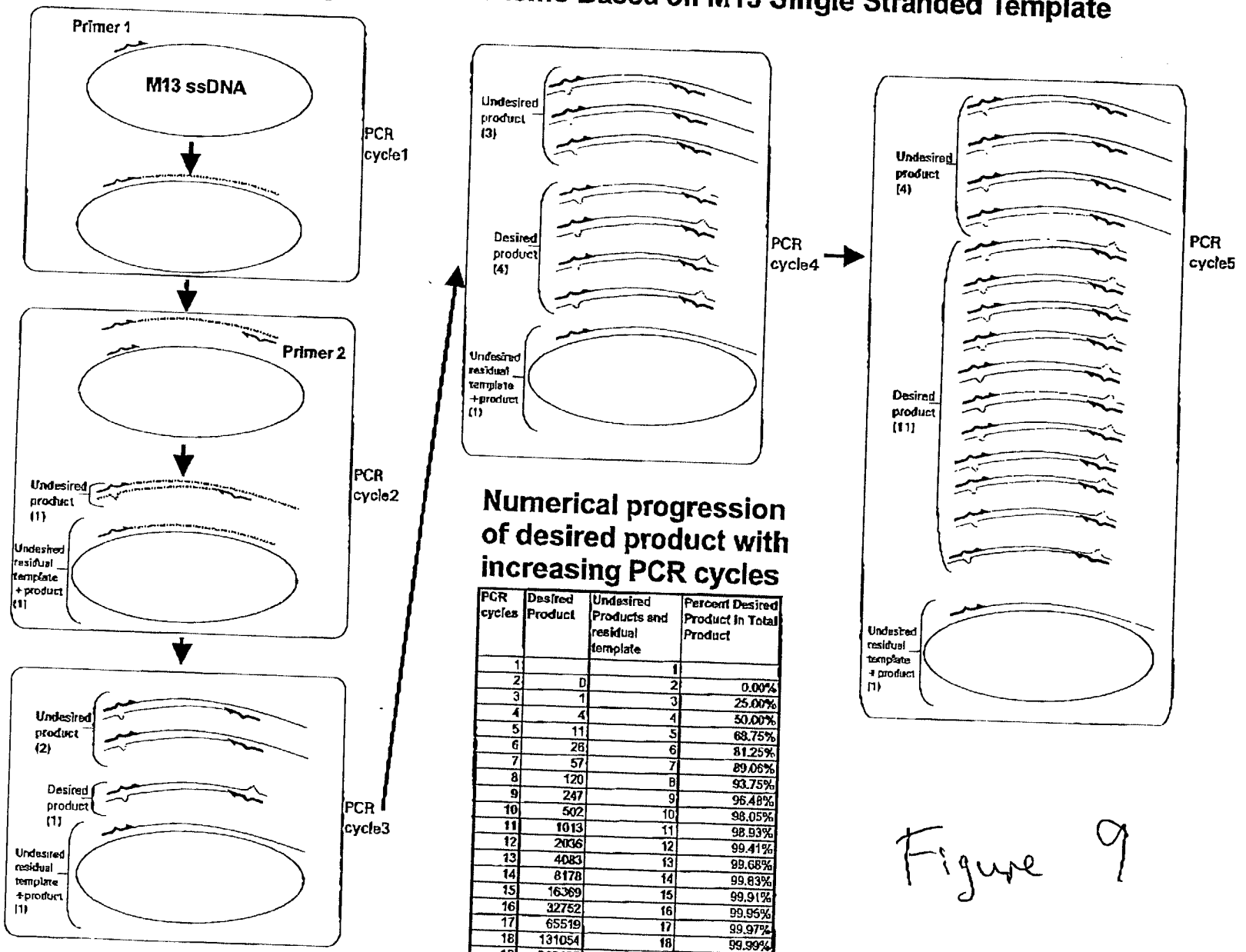
F1 + ~~B~~ R3

Diagram 3

Fig 8



Amplification Scheme Based on M13 Single Stranded Template



Numerical progression of desired product with increasing PCR cycles

PCR cycles	Desired Product	Undesired Products and residual template	Percent Desired Product in Total Product
1		1	
2	0	2	0.00%
3	1	3	25.00%
4	4	4	50.00%
5	11	5	68.75%
6	26	6	81.25%
7	57	7	89.06%
8	120	8	93.75%
9	247	9	96.48%
10	502	10	98.05%
11	1013	11	98.93%
12	2036	12	99.41%
13	4083	13	99.68%
14	8178	14	99.83%
15	16369	15	99.91%
16	32752	16	99.95%
17	65519	17	99.97%
18	131054	18	99.99%
19	262125	19	99.99%
20	524268	20	100.00%

Figure 9